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Factors Affecting Primary Stability on Sites of Alveolar Ridge Preservation Using Porcine-derived Bone Minerals

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Purpose: The alveolar ridge preservation (ARP) is widely conducted for implant placement. However, experimental results using deproteinized porcine bone mineral (DPBM) have been scarce. This retrospective study evaluated factors affecting the primary stability of implants in an area where ARP was performed using DPBM.

Materials and Methods: Thirty-eight patients were divided into two groups based on the primary stability, with torque value of 30 Ncm as borderline. To determine the factors that affect the primary stability of implants, we collected data from patients' medical records including age, sex, reentry time, socket location, remaining bone wall at the time of extraction, and type of collagen membrane, as well as from radiographs and histomorphometric analysis.

Result: The results showed statistically significant difference for the remaining extraction socket wall (P=0.014), residual graft (P=0.029), and fibrovascular tissue (P=0.02) between the two groups. There was an insignificant tendency toward the time of reentry surgery (P=0.052) and location (P=0.077). All implants placed in sites using DPBM functioned well up to 3 years.

Conclusion: Within the limitations of the present study, extraction socket wall, residual graft, and fibrovascular tissue can affect the primary stability at the time of implant placement on grafted sites using DPBM and collagen membranes. In addition, reentry time and locations can be considered. In future studies, comparative experiments in quantified models will be required to supporting the findings.

Key Words: Alveolar ridge augmentation; Dental implant; Xenograft

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Introduction

Dimensional changes in the alveolar ridge following tooth extraction are inevitable. The buccal plates of the post-extraction site undergo extensive horizontal bone loss, while there are slight vertical changes[1]. Thus, a lingual/palatal shift of the alveolar ridge occurs. As these dimensional changes are unfavorable for the aesthetics and placement of implants, clinicians consider alveolar ridge preservation (ARP) to avoid these bony changes.

Several studies have shown that ARP has an advantage in reducing the shrinkage of the alveolar ridge after tooth extraction compared to the untreated socket although it could not terminate the resorption of the alveolar ridge[2-6]. Specifically, these studies observed a significant reduction in ridge width loss, and preservation or even occasional growth of ridge height in the ARP group have been reported.

Although several studies on ARP have focused on the results of the dimensional change of the alveolar ridge and its histological characteristics based on different materials and surgical techniques, there has scarcely been research on its osseointegration ability and contributing factors during implant placement. Moreover, experimental results using porcine bone (deproteinized porcine bone mineral, DPBM)[7,8] compared with autogenous bone[9-11], allografts[12-14], xenografts (specifically deproteinized bovine bone mineral)[15-18], and alloplastic bone[19-22] and those with or without membranes[23,24] have been relatively rare. Therefore, our study evaluated the factors related to primary stability during implant placement in ARP sites using DPBM and try to find the clinical relevance.

Materials and Methods

This retrospective study was conducted to evaluate the factors affecting the primary stability of implants in areas where ridge preservation was performed using DPBM. The Institutional Review Board of Veterans Health Service Medical Center reviewed and approved the protocol for this retrospective study (BOHUN 2016-12-002).

1. Patient Population

Thirty-eight patients who visited the Department of Periodontology of Veterans Health Service Medical Center between January 2015 and December 2016 were selected in this study. From the total number of patients (n=38), 5 patients with non-resorbable membranes, 2 patients with smoking history, and 2 patients with abnormalities in the box plot of residual graft and fibrovascular tissue were excluded. Finally, 29 remaining patients were included in the statistical analysis (Fig. 1). Patients were divided into two groups (Group A, primary stability ≥30 Ncm; Group B, primary stability <30 Ncm) based on the primary stability at the time of implant placement using a manual torque wrench (Fig. 2).

2. Surgical Procedure

Following tooth extraction, the mucoperiosteal flap was raised and complete debridement was performed. The patients received the ridge preservation procedure using DPBM (THE Graft; Purgo, Seoul, Korea) and a collagen membrane (Ossix-plus; Datum, Telrad, Israel, or Bio-Gide; Geistlich Pharma AG, Wolhusen, Switzerland). The buccal flap was released, achieving primary closure with 5-0 monofilament nylon suture (BLUE NYLON; Ailee Co., Ltd, Busan, Korea). Postoperative antibiotics were prescribed for 7 days. After the healing period (mean 24.2±13.04 weeks, range 9~86), a trephine bur (Trephine Bur Kit Xit; Dentium, Seoul, Korea) with a 3.0-mm outer diameter was used to harvest the bone for histomorphometric analysis. The implants were subsequently placed on the grafting site, and the primary stability was measured by the insertion torque resistance analysis method (Fig. 3).

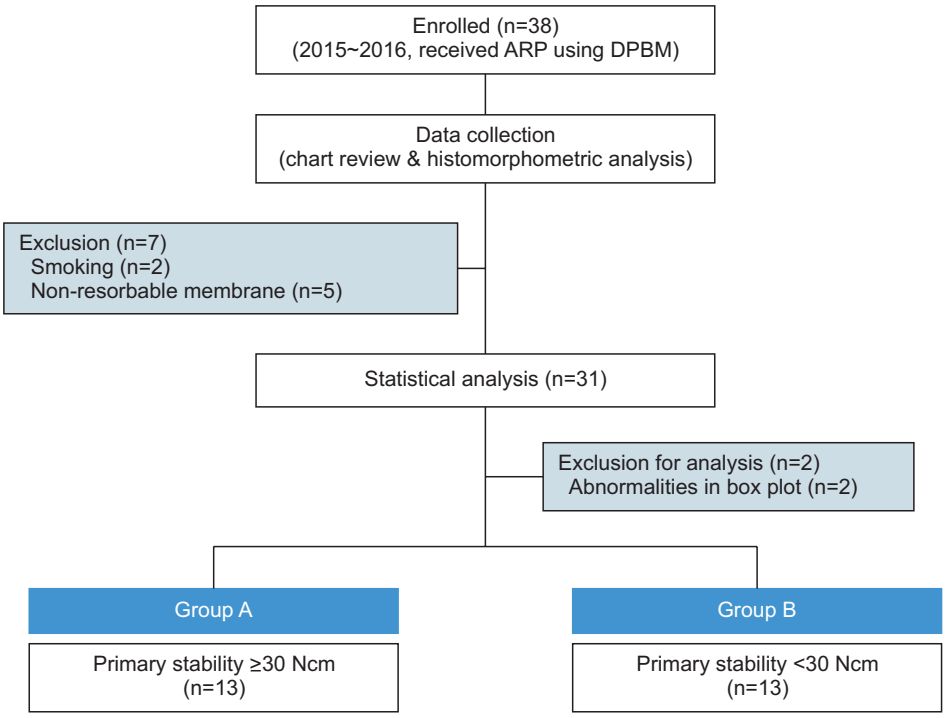

Fig. 1. Study flow. ARP: alveolar ridge preservation, DPBM: deproteinized porcine bone mineral.

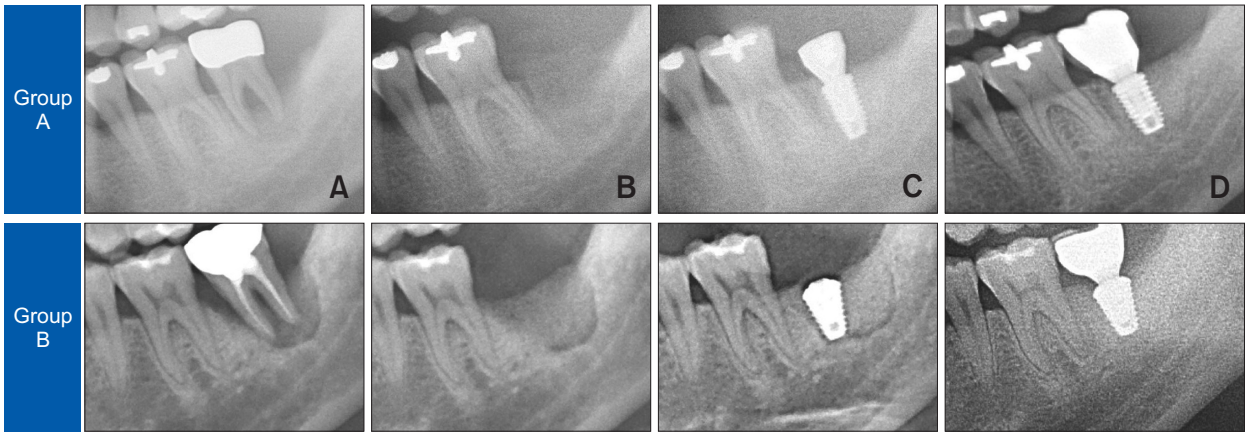

Fig. 2. Treatment process according the primary stability at the time of implant placement. (A) Before extraction. (B) After extraction and grafting. (C) Implant placement. Depending on primary stability, healing abutment (Group A) or cover screw (Group B) was connected, and (D) follow-up after l-year loading. Group A: primary stability ≥30 Ncm, Group B: primary stability <30 Ncm.

3. Data Collection

To determine the factors that affect the primary stability of implants, we collected data from the patients' chart review, radiographs, and histomorphometric analysis. Patient charts included information on age, sex, reentry time, socket location, type of membrane, and the number of remaining bone walls (< or =3-mm hard tissue loss in height) at the time of extraction. Histomorphometric analysis was performed with the biopsy core samples that were fixed in 10% buffered neutral formalin (Sigma Aldrich, St. Louis, MO, USA) for 14 days. Subsequently, the bone cores were decalcified in 5% formic acid and embedded in paraffin. The paraffin block was trimmed using a rotary microtome (Leica RM2135; Leica Biosystems, Seoul, Korea) to a thickness of about 300 um until the central surface of the tissue appears. Subsequently the final tissue section with a thickness of 4 um was

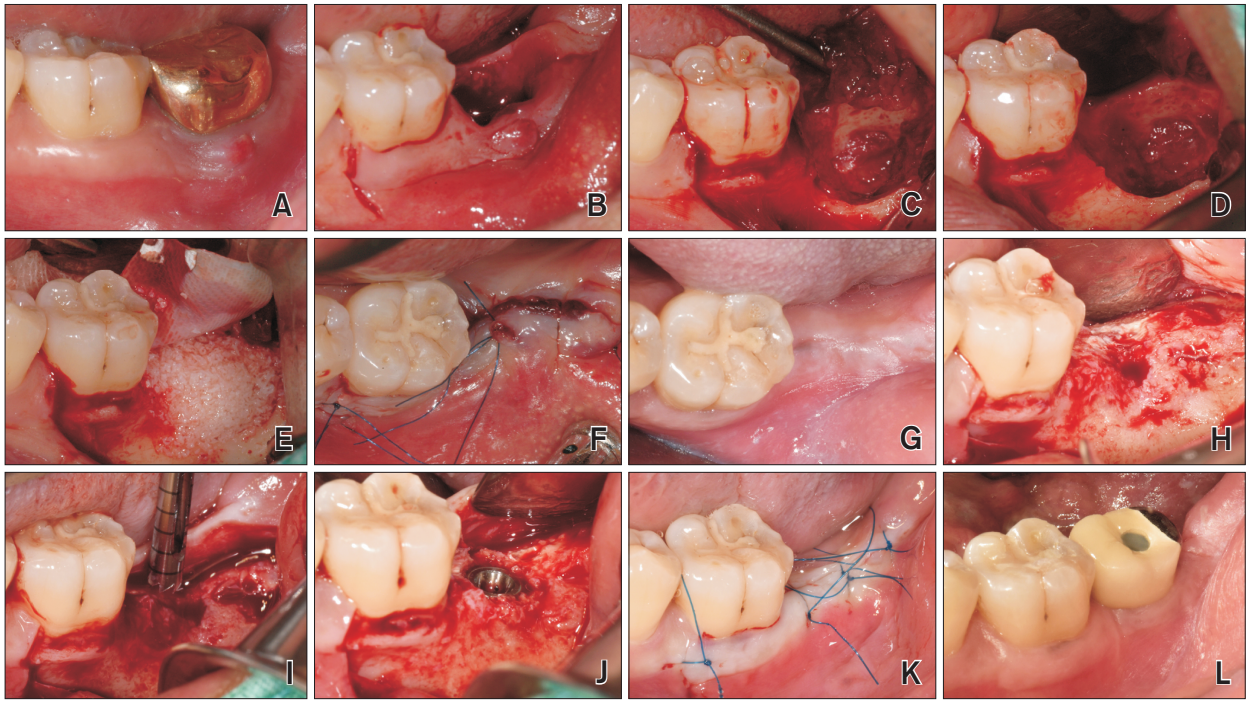

Fig. 3. Surgical procedure and final restoration of a representative case in Group B. (A) Before extraction. (B) Vertical incision after extraction. (C) Detachment of granulation tissue. (D) Checking the extraction socket wall. (E) Grafting porcine mineral and membrane. (F) Suturing. (G) Before reentry. (H) Flap reflection for implant placement. (I) Core biopsy. (J) Implant placement (primary stability was less than 30 Ncm). (K) Suturing. (L) Final restoration. Group B: primary stability <30 Ncm.

attached to the coated slide glass. After drying for 1 hour in a dry oven, hematoxylin and eosin (H&E) dyeing was performed, and the central-most sections were stained with H&E. The area and percentage of new bone, residual bone graft material, and fibrovascular tissue were calculated using the imaging program (Image-Pro Plus 7; Media Cybernetics Inc., Rockville, MD, USA) as previously described[25]. Radiographic evaluation was performed to evaluate the marginal bone loss by periapical radiographs at the time of the final prosthesis installation (baseline) and at 1 year. The difference of between baseline and 1 – year follow-up of distance (abutment – bone level) of both mesial and distal area was calculated. A digital caliber (mViewer; Marotech, Seoul, Korea) was used to measure the radiographs taken by a long-cone paralleling technique and a film holder (XCP-DS FIT; Dentsply Sirona, Charlotte, NC, USA)[26].

4. Statistical Analyses

Continuous variables were expressed as mean± standard deviation (SD) or median (interquartile range) based on normality. Categorical variables were expressed as frequencies and percentages. Fisher's exact test for categorical variables was used. The t-test for continuous variables with normality and the Mann–Whitney U-test for continuous variables without normality were used to assess the differences between groups. Normality tests were performed using the Shapiro–Wilk test. All statistical analyses were performed using R 3.6.3 (R Development Core Team; R Foundation for Statistical Computing, Vienna, Austria). P-values <0.05 were considered statistically significant.

Result

1. Patient Population

Group A (n=13) comprised patients with primary

stability greater than 30 Ncm, whereas Group B (n=16) comprised patients with primary stability less than 30 Ncm. The mean ages of Groups A and B were 62.08±10.24 and 61.12±10.90 years, respectively. Group A comprised nine male and four female, whereas Group B comprised nine male and seven female. Age and sex had no statistical relevance to primary stability (Table 1).

2. Socket Location

The distribution of the socket location was as follows: maxillary premolars (n=2, 15.38%), maxillary molars (n=1, 7.69%), mandibular premolars (n=3, 23.08%), and mandibular molar (n=7, 53.85%) in Group A and maxillary premolars (n=4, 25.00%), maxillary molars (n=6, 37.50%), mandibular molars (n=6, 37.50%), and no mandibular premolars in Group B. There was no statistically significant association between socket location and primary stability,

Table 1. Patient demography according to the primary stability group

Variable	Group A (n=13)	Group B (n=16)	P-value
Age (yr)	62.08±10.24	61.12±10.90	0.812
Sex			0.702
Male	9 (69.23)	9 (56.25)	
Female	4 (30.77)	7 (43.75)	
Socket location			0.077
Mx. premolar	2 (15.38)	4 (25.00)	
Mx. molar	1 (7.69)	6 (37.50)	
Mn. premolar	3 (23.08)	0 (0.0)	
Mn. molar	7 (53.85)	6 (37.50)	
Membrane type			0.192
Cross linked	5 (38.46)	2 (12.50)	
Non cross linked	8 (61.54)	14 (87.50)	
Re-entry time			0.052
≤20 weeks	2 (15.38)	9 (56.25)	
>20 weeks	11 (84.62)	7 (43.75)	
Remaining wall			0.014*
1 wall	0 (0.0)	1 (6.25)	
2 walls	4 (30.77)	12 (75.00)	
3 walls	8 (61.54)	2 (12.50)	
4 walls	1 (7.69)	1 (6.25)	
Histomorphometric results			
Residual graft	17.12±9.35	10.20±6.85	0.029*
New bone	8.55 [7.81-24.35]	6.28 [2.17-13.43]	0.263
Fibrovascular tissue	67.88±8.45	78.74±13.87	0.020*
Marginal bone loss			
Mesial bone	0.01 [0.01-0.02]	0.02 [0.01-0.04]	0.292
Distal bone	0.01 [0.01-0.02]	0.01 [0.00-0.04]	0.700

Group A: primary stability ≥30 Ncm, Group B: primary stability <30 Ncm, Mx.: maxillary, Mn.: mandibular.

Continuous data are expressed as mean±standard deviation or median [quartile range], and P-values were calculated by the t-test or Mann-Whitney U-test based on normality.

Categorical data are expressed as frequencies and percentages, and Fisher's exact test calculated the P-values.

*P<0.05.

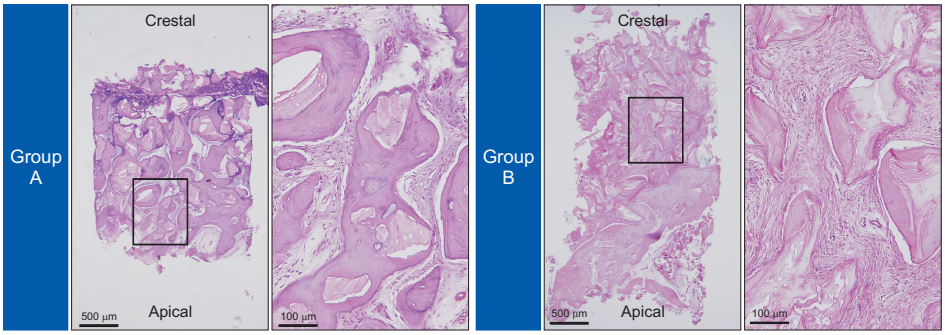

Fig. 4. Histologic views of representative specimen in Group A and Group B. These slides were stained by hematoxylin and eosin (H&E). Group A: primary stability ≥30 Ncm, Group B: primary stability <30 Ncm.

as the P-value was 0.077. However, a slight tendency toward significance was observed.

3. Type of Membrane

Two types of membranes were introduced in this study: non-crosslinked (Bio-Gide; Geistlich Pharma AG) and crosslinked collagen membranes (Ossix-plus; Datum). Group A comprised 5 crosslinked and 8 non-crosslinked membranes, and Group B comprised 2 crosslinked and 14 non-crosslinked membranes. There was no statistically significant difference between the two groups (P=0.192).

4. Reentry Time

Based on a reentry time of 20 weeks, in Group A, the reentry times were 20 weeks or less in 2 (15.38%) patients and >20 weeks in 11 (84.62%) patients. In Group B, the reentry times were 20 weeks or less in 9 (56.25%) patients and >20 weeks in 7 (43.75%) patients. The P-value was 0.052, and although there was no statistical significance, there was a slight trend between the reentry time of 20 weeks and primary stability.

5. Remaining Extraction Socket Wall

In Group A, two-, three-, and four-wall defects were observed in 4 (30.77%), 8 (61.54%), and 1 (7.69%) patients, respectively, and none of the patients had a one-wall defect. In Group B, one-, two-, three-, and four-wall defects were observed in 1 (6.25%), 12 (75.00%), 2 (12.50%), and 1 (6.25%) patients, respectively. There was a statistically significant difference between the two groups (P=0.014).

6. Histomorphometric Analysis

In 29 specimens, various patterns of bone formation were observed. Osteoconductive graft material and intimate new bone formation were observed in specimens with satisfactory bone formation. Bone anastomosis between these de novo bones was also observed. The bone anastomosis thickened over time and settled, and loose soft marrow tissue was observed to fill. Although some specimens showed several different kinds of bone formation, inflammation patterns were rarely observed (Fig. 4).

The biopsy core sample was divided into three groups: new bone, residual graft, and fibrovascular tissue. In Group A, the mean values±SD of the new bone, residual graft, and fibrovascular tissue were 14.99%±13.36%, 17.12%±9.35%, and 67.88%±8.45%, respectively. In Group B, the mean values of the new bone, residual graft, and fibrovascular tissue were 11.07%±14.85%, 10.20%±6.85%, and 78.74%±13.87%, respectively. Residual graft (P=0.029) and fibrovascular tissue (P=0.02) showed statistical significance to the primary stability; however, there was no statistically significant difference in the new bone between the two groups (P=0.465).

7. Radiographic Evaluation: Marginal Bone Loss

In each group, marginal bone loss was measured 1 year after implant prosthesis delivery. Using periapical radiographs, the alteration of mesial/distal height was evaluated. In Group A, the loss of both

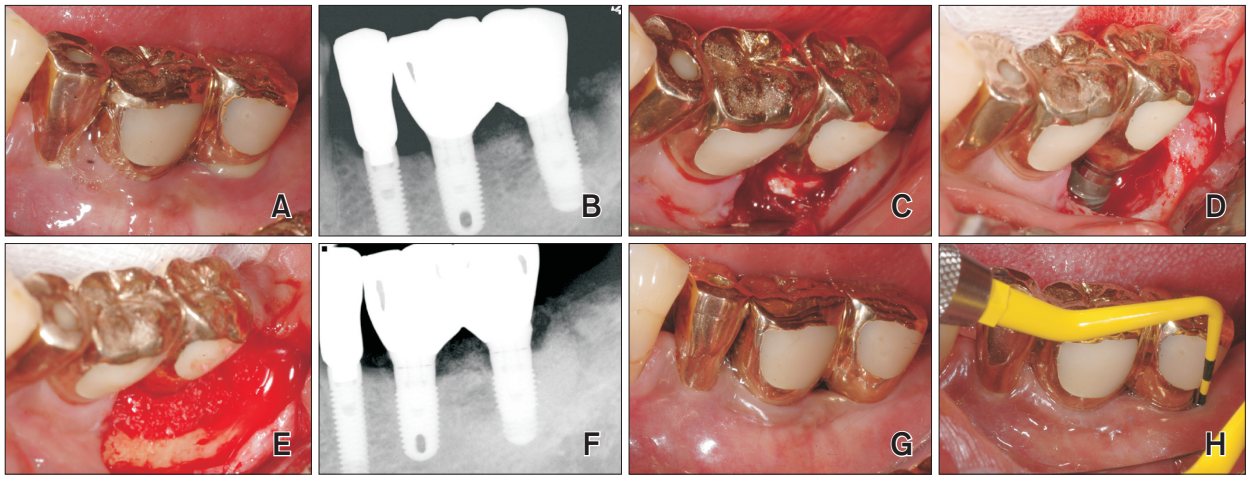

Fig. 5. A case of eventful follow-up in Group B. (A) Pus discharge on the left 2nd molar. (B) Periapical radiograph. (C) Excessive cement was found after flap reflection. (D) Debridement. (E) Grafting of the porcine mineral. (F) Periapical radiograph at 2-year follow-up. (G) No symptom and eventful finding at 2-year follow-up. (H) Clinical photo showed improved clinical parameters (within normal probing depth and no bleeding). Group B: primary stability <30 Ncm.

mesial and distal bone height was the median value of –0.01 mm (interquartile range, 0.01~0.02). In Group B, the median mesial height was 0.02 mm (interquartile range, 0.01~0.04), and the distal height was –0.01 mm (interquartile range, –0.04~0). There was no statistically significant difference in either the mesial or distal wall (mesial height: P=0.292, distal height: P=0.7). However, one site in Group B showed abundant bone resorption and pus discharge 6 months after implant placement. After the flap was elevated, the shape of the defect was judged as a defect limited to the buccal area associated with excessive cement. Therefore, detoxification using tetracycline and additional bone grafting using DPBM and collagen membrane were performed. This implant functioned well until the final observation after 2 years (Fig. 5).

Discussion

This retrospective study showed statistically significant association with the following: (1) residual graft, (2) fibrovascular tissue (connective tissue, vessel), and (3) remaining extraction socket wall between the two groups, which were divided according to the primary stability at implant placement on ARP sites with DPBM and collagen membrane. Additionally, there was a slight tendency towards significance in reentry time and socket location, whereas there was no statistical significance in age, sex, marginal bone loss, and type of membrane.

Based on the result of histomorphometric analysis from our data, the residual graft and fibrovascular tissue were associated with primary stability. In the sites where more residual graft material was present and in the sites where less soft tissue was present, higher the primary stability of the implant was observed. However, the meaning of the residual graft is questionable since some studies have emphasized its negative effect as the presence of residual graft interferes with the normal healing process[2,4,12,16]. However, there was a slight discrepancy between histological and clinical findings[27]. These issues may remain as future research topics. Detailed histological analysis of the remaining graft material, *i.e.*, a new bone or an existing remnant bone, a connection of the remaining graft material, and an overall distribution, requires more analysis in a quantitative research model rather than a retrospective study showing heterogeneous diversity. These histological findings with connectivity and distribution would

be more likely to affect the primary stability than the percentage from histomorphometric results.

From our data, we can determine the association between the number of extraction socket walls and primary stability. As the number of remaining socket walls increased, the primary stability tended to increase statistically significantly. As the primary stability is achieved by mechanical engagement, the number of bone walls has a positive impact on its stability. Moreover, it seems to be associated with greater regeneration capacity from the large number of remaining bone walls.

Although there was no significance in socket locations, the maxillary molar area which comprises lower bone density shows a tendency of slightly lower primary stability and the mandibular premolar had higher primary stability. The osseointegration is achieved in two stages with primary stability from the mechanical engagement of the cortical bone and secondary stability from the bone formation around the implant[28]. In this report, the bone density known as the ratio of trabecular/cortical bone contributes to primary stability. In addition, the socket location of the implant is relevant to primary stability[29].

In our study, reentry time after ARP showed another slight tendency. In previous studies, the healing time was considered the period when minimal volumetric change was observed, 4~6 months of healing time was selected[12,30]. Moreover, reentry time was determined based on bone quality. Therefore, 6 weeks in D2, 4 months in D3, and longer periods than 6 months in D4 were recommended[2]. However, according to a recent review article including histological modification, healing periods, subsequent reentry for implant placement, and longer healing time were not mandatory because reentry is possible in case where no more physiological acceleration of the healing process through the amount of change in bone and connective tissue area is presented[31]. Additionally, they explained that the reentry time of 3 and 5 months was determined according to the residual grafts of allograft and xenograft. In our study, although there was a slight tendency toward significance between the reentry time and the primary stability, the primary stability tended to increase at 21 weeks, which might be associated with a histological modification of the DPBM.

In this study, two types of membranes with carrying characteristics were used. It is known that there is a low risk of soft tissue dehiscence and infection with non-crosslinked collagen membranes. However, it is rapidly absorbed by collagenase, resulting in a short duration. A crosslinked membrane was developed to overcome this disadvantage of non-crosslinked membranes. By increasing the crosslinking between collagen fibers, the duration time was increased. However, poor operability was noted because of its stiffness compared to the non-crosslinked membrane. Similar to other comparative studies, it was concluded that there was no difference in volumetric changes when comparing the two membranes in the case of dehiscence. In this study, it seems that the type of membrane did not contribute significantly[32]. Moreover, radiographic evaluation showed that all of the values of marginal bone loss met the implant success criteria of less than 0.2-mm marginal bone loss after 1 year. Although one patient had peri-implantitis due to the remnant of excess cement around the cement-retained implant restoration[33], it could be concluded that ARP using porcine bone is a successful treatment technique similar to other materials[2,3,5,31,34,35].

Our study has several limitations. First, this study used small sample size and was considered as an uncontrolled study, considering its retrospective study. Additionally, we did not evaluate the volumetric changes in the ARP-treated sites, and other factors that could affect the primary stability were not included in this study, such as the thickness of the buccal bone. There were cases where it was difficult to distinguish bone wall defects that depended on the chart. The histological evaluation showed the exis-

tence of a broad range value. These limitations have also been shown in other studies[36]. Although lack of standardization was attributed to the heterogeneity of the results, our study contributes to clinical findings by focusing on the association between the ARP techniques and implant stability. Additionally, several studies restricted their data based on the intact extraction socket or damaged socket, which has more than three walls to obtain elaborate results[37]. In contrast, our study included more damaged extraction sockets, which is expected to result in severe bone loss if left untreated.

According to various variables, the primary stability may have been affected when the implant was placed in the site where the alveolar graft was performed. In this study, all implants that were placed in sites using DPBM functioned well for up to 3 years, regardless of whether the primary stability was low or high. It may not be an essential requirement for the success of an implant[38]. However, primary stability at the time of implant placement is important to perform one-staged surgery without uncovering surgery to connect the healing abutment for clinicians and patients.

Conclusion

Within the limitations of the present study, extraction socket wall, residual graft, and fibrovascular tissue were presumed to affect the primary stability at the time of implant placement on grafted sites using DPBM and collagen membranes. In addition, reentry time and locations can be considered. In future studies, comparative experiments in quantified models will be required for supporting these parts.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgement

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